RELATIONSHIP BETWEEN BIOCHEMICAL, SEROLOGICAL, HISTOLOGICAL AND VIRAL REPLICATION IN ASYMPTOMATIC HBsAg POSITIVE INDIVIDUALS

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CERTIFICATE

COIMBATORE MEDICAL COLLEGE & HOSPITAL

CERTIFICATE

This is to certify that the Dissertation entitled **"RELATION BETWEEN BIOCHEMICAL, SEROLOGICAL, HISTOLOGICAL AND VIRAL REPLICATION INDICES IN ASYMPTOMATIC HBsAg POSITIVE INDIVIDUALS,** submitted by **Dr. Sathi.V,** Post-Graduate in General Medicine, Coimbatore Medical College, to The Tamilnadu Dr. M.G.R. Medical University is a record of a bonafide research work carried out by him under my guidance and supervision from January 2007 to June 2009.

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DECLARATION

I solemnly declare that the Dissertation titled **"RELATION BETWEEN BIOCHEMICAL, SEROLOGICAL, HISTOLOGICAL AND VIRAL REPLICATION INDICES IN ASYMPTOMATIC HBsAg POSITIVE INDIVIDUALS ",** was done by me at Coimbatore Medical College & Hospital during the period from September 2007 to September 2009 under the guidance and supervision of my unit chief Prof. Dr .P. JAMBULINGAM.

This dissertation is submitted to The Tamilnadu Dr. *M.G.R.* Medical University towards the partial fulfillment of the requirement for the award of M.D. Degree (Branch *I*) in General Medicine – March 2010

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INTRODUCTION

INTRODUCTION

Hepatitis B (formerly known as "serum" hepatitis) is an acute systemic infection with major pathology in the liver, caused by hepatitis B virus (HBV) and transmitted usually by the parenteral route. It is clinically characterised by a tendency to a long incubation period (6 weeks to 6 months) and a protracted illness with a variety of outcomes. Usually, it is an acute self-limiting infection, which may be either subclinical or symptomatic. In approximately 5 to 15 per cent of cases, HBV infection fails to resolve and the affected individuals then become persistent carriers of the virus. Persistent HBV infection may causes, progressive liver disease including chronic active hepatitis and hepatocellular carcinoma. There is also evidence of a-close association between hepatitis B and primary liver cancer Hepatitis B virus can form a dangerous alliance with delta virus and produce a new form of virulent hepatitis which is considered to be a widespread threat for much of the world.

Hepatitis B is endemic throughout the world, especially in tropical and developing countries and also in some regions of Europe. Its prevalence varies from country to country and depends upon a complex

mix of behavioural, environmental and host factors. In general, it is lowest in countries or areas with high standards of living.

The HBV infection is a global problem, with 66 percent of all the world's population living in areas where there are high levels of infection.

More than 2 billion people worldwide have evidence of past or current HBV infection and 350 million are chronic carriers of the virus, which is harboured in the liver, the virus causes 60-80 per cent of all primary liver cancer, which is one of the three top causes of cancer death in East the Pacific Basin and Sub -Saharan Africa..

Infection with HBV is a major cause of morbidity and mortality in the SEAR. More than One-third of the population has been infected with HBV and it is estimated that there are 80 million HBV carriers (about 6% of the total population). Many are lifelong carriers, although not all are infectious, and some will clear the virus after intervals varying from many months to years. Between 5 percent and 10 percent of adults and up to 80 per cent .of infants infected with HBV will become carriers. Among these, 25 per cent will, in the long term, develop serious liver disease. Approximately 14 to 16 million people are infected with HBV each year in the region. It is estimated that approximately 2 lakh deaths associated

with acute HBV infection and related disease occur in the Region annually.

Based on the different HBsAg carrier rates, countries of the Region can be divided into three epidemiological patterns. The Type 1 occurs in Nepal and Sri Lanka and is characterized by a low HBsAg carrier rate of 0.9 to 1.0 per cent. The second pattern (Type 2) can be found in Bhutan, India, Indonesia and Maldives where carrier rate is high in the general population (5 to 7 per cent). In India alone there are an estimated 43 to 45 million HBsAg carriers and, among them 10 to 12 million also have HBeAg. Type 3 is observed in Bangladesh, DPR Korea, Myanmar and Thailand, where the carrier rate is very high and ranges from 9 per cent to 12 per cent.

In India, the carrier rate of HBsAg in hospital staff has been found to be higher (10.87 per cent) than in voluntary blood donors (6 per cent) and in the general population (5 per cent).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Hepatitis B Virus is a DNA virus with remarkably compact genomic structure, despite its small, circular, 3200 – bp size, HBV DNA

codes for four sets of viral products with a complex, multiparticle structure. HBV achieves its genomic economy by relying on an efficient strategy of encoding portents from four overlapping genes. S,C, P and X.

HBV is recognized as one of our family of animal viruses, hepadna virueses and is classified as hepanda virus type – 1.

HBV has three distinctive three morphological forms. The most numerous are the 22nm particles, which appear as spherical or long filamentous forms; these are antigenically indistinguishable from the outer surface are envelope protein of HBV and are thought to represent excess viral envelope protein.

The envelope protein expressed on the outer surface of the virion and on the smaller spherical and tubular structures is referred to as hepatitis B surface antigen (HBsAg). The envelope protein HBsAg is the product of S gene of HBV.

The intact 42-nm virion contains 27 –nm nucleocapsid core particle. Nucleocapsid proteins are coded for by the C gene, The antigen expressed on the surface of the nucleocapsid core is referred to as hepatitis B core antigen (HBcAg) and its corresponding antibody is anti – HBc.

A third HBV antigen is hepatitis B e antigen (HBeAg), a soluble, nonparticulate, nucleocapsid protein that is immunologically distinct from intact HBcAg but is a product of the same C gene. The C gene has two initiation codons, a precore and a core region. If translation is initiated at the precore region, the protein product is HBeAg, which has single peptide that binds it to the smooth endoplasmic reticulum and leads to its secretion into the circulation. If translation begins with the core region, HBcAg is the protein product; it has not single peptide, it is not secreted, but it assembles into nucleocapsid particles, which bind to incorporate RNA and which, ultimately contain HBV DNA.

HBcAg particles remain in the hepatocyte, where they are readily detectably by immunohistochemical staining, and are exported after encapsidation by an envelope of HBsAg. Therefore, naked core particle do not circulate in the serum. The secreted nucleocapsid protein. HBeAg, provides a convenient, readily detectable, qualitative marker of HBV replication and relative infectivity.

HBsAg – positive serum containing HBeAg is more likely to be highly infectious and to be associated with the presence of hepatitis B virions than HBeAg – negative or anti HBe – Positive serum.

Early during the course of acute hepatitis B, HBeAg appears transiently, its disappearance may be a harbinger of clinical improvement and resolution of infection. Persistence of HBeAg in serum beyond the first 3 months of acute infection may be predictive of the development of chronic infection and the presence of HBeAg during chronic hepatitis B is associated with ongoing viral replication, infectivity, and inflammatory liver injury.

The third of the HBV genes is the largest, the P gene which codes for the DNA polymerase. This enzyme has both DNA dependent DNA polymerase and RNA dependent reverse transcriptase activities. The fourth gene, X, codes for a small, non-particulate protein, hepatitis B x antigen (HBxAg).

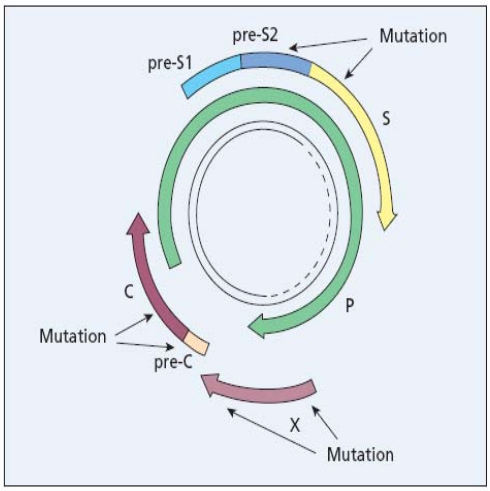


Fig. 17.5. The site of HBV mutations.

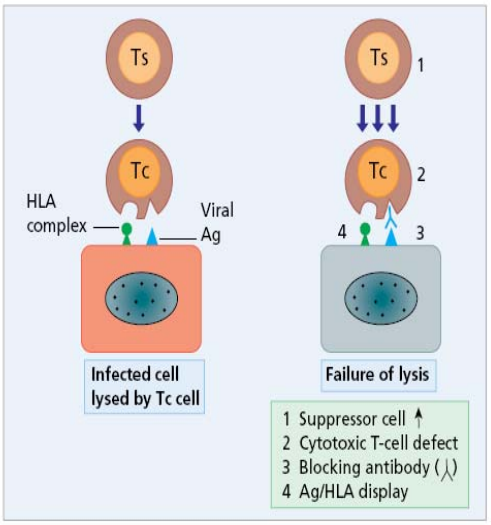


Fig. 17.7. T-lymphocyte lysis of infected hepatocytes and mechanisms of failure of lysis in chronic hepatitis. Tc, cytotoxic cell; Ts, suppressor cell.

HBsAg major protein

TRANSMISSION

HBV is parenterally transmitted via blood or blood products or by sexual or perinatal exposure, the same routes as for HIV; hence, there are many similarities in the epidemiology of these two viruses. Viral particles, which are capable of transmitting infection to chimpanzees, are detectable in body secretions, including semen and saliva. Thus, contact with mucous membranes and their secretions is likely to be a mode of transmission of HBV.

PERINATAL AND EARLY CHILDHOOD TRANSMISSION

HBV is most prevalent in people born in regions of high HBV endemism and their descendants. High levels of virus in serum (signified by HBV DNA and HBeAg positivity) have been associated with an increased risk of transmission by needle stick exposure and by vertical routes. Infants born to HBeAg-positive mothers who have high levels of viral replication (HBV DNA level >80 pg/mL) have a 70% to 90% risk of perinatal acquisition in the absence of interventions. In contrast, the risk of mother-to-infant transmission from HBeAg-negative mothers is substantially lower (10%–40%). Infection occurs through occult inoculation of the infant at the time of birth or shortly thereafter. IgM anti-HBc is not detectable in cord blood, so that intrauterine infection is unlikely to have occurred. Even with active and passive immunization, 5% to 10% of babies may acquire HBV infection at birth.

Children of HBsAg-positive mothers who are not infected at birth remain at high risk of early childhood infection; 60% become infected by the age of 5 years.The mechanism of this later infection, which is neither perinatal nor sexual, is unknown. Although HBsAg can be detected in breast milk, breast-feeding is not believed to be an important mode of transmission. Children living in areas of high endemism may acquire infection outside the family.

SEXUAL TRANSMISSION

Sexual activity is probably the single most important mode of HBV transmission in areas of the world such as North America, where the prevalence of infection is low. From 1980 to 1985, men who had sex with men were at particularly high risk of HBV infection and accounted for 20% of all reported cases of HBV infection. Factors associated with a high risk of viral acquisition in this patient population included multiple sexual partners, anal-receptive intercourse, and duration of sexual activity. The risk has fallen markedly in recent reports (to 8% of all cases), probably because of modifications of sexual behavior in response to the acquired immunodeficiency syndrome (AIDS) epidemic. Unfortunately, advanced liver disease from HBV infection is emerging as an important medical problem for patients infected with HIV (see later).

Heterosexual sex now accounts for the majority of cases of HBV infection (26%) with an identifiable risk factor in the United States. In heterosexuals, factors associated with an increased risk of HBV infection include duration of sexual activity, number of sexual partners, a history of sexually transmitted diseases, and positive serologic results for syphilis. Sexual partners of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk of HBV infection.

Sexual partners of persons infected with HBV are at risk for infection, even in the absence of high-risk behavior. Studies of sexual and household contacts of HBV carriers have shown that 0% to 3% of the spouses or sexual partners and 4% to 9% of the children are HBsAg positive. Moreover, there is a high prevalence of markers of prior HBV infection in these two groups (29%–59% of spouses or sexual contacts and 9%–12% of children). Because many patients with chronic HBV infection are unaware of their infection and are "silent carriers," sexual transmission is likely to be an important mode of transmission worldwide. As with perinatal transmission, sexual transmission is facilitated by active viral replication in the infected person. The risk of heterosexual transmission is greater when the infected person is female than when the infected person is male. The use of condoms appears to reduce the risk of sexual transmission. Sexual transmission may also account for some of the approximately 30% to 40% of all cases in the United States in which no known risk factor can be identified.

INJECTION DRUG USE

In the United States and Western Europe, injection drug use remains a very important mode of HBV transmission (23% of all cases). The risk of HBV infection increases with duration of drug use, so that serologic markers of ongoing or prior HBV infection are almost universal after 5 years of drug use.

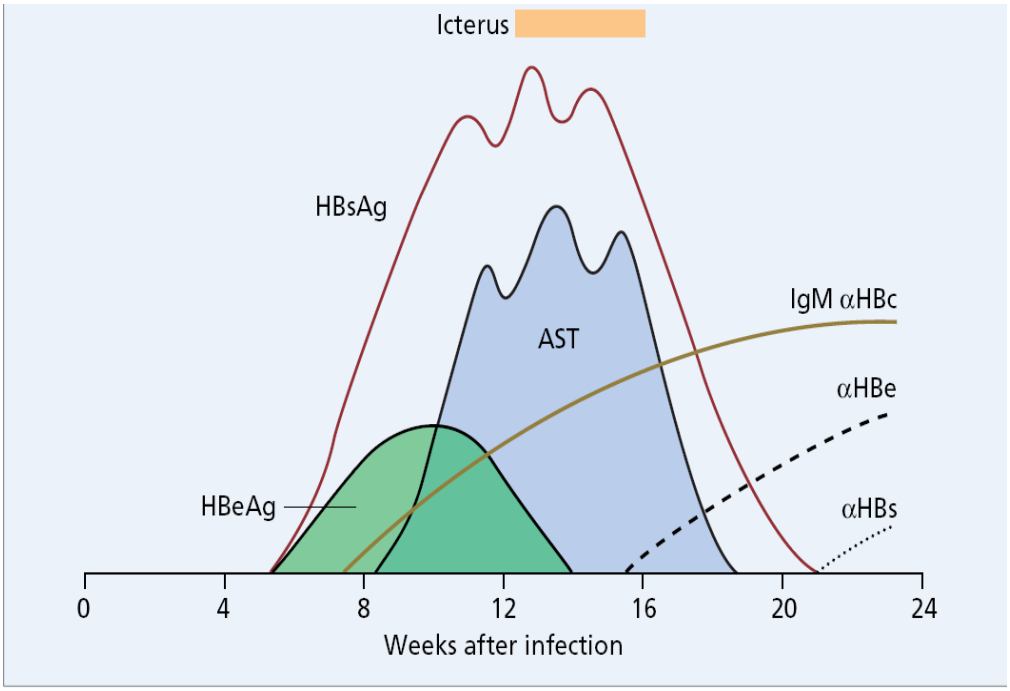
OTHER MODES OF TRANSMISSION

Other risk factors for HBV infection include working in a health-care setting (3% of cases in the United States), transfusions and dialysis (1% each), acupuncture, tattooing, travel abroad, and residence in an institution. Although the risk of transfusion-associated HBV infection has been greatly reduced with the screening of blood (with tests to detect HBsAg and anti-HBc) as well as exclusion of donors who engage in high-risk activities, it is estimated that 1 in 50,000 transfused units transmits HBV infection. Acupuncture has been associated with outbreaks of HBV infection. In World War II, contaminated plasma-stabilized yellow-fever vaccine was associated with one of the largest epidemics of HBV infection, in which icteric hepatitis developed in 50,000 U.S. army personnel. Nosocomial spread of HBV infection in hospitals, particularly in dialysis units as well as in dental units, has been well described, even when current infection control practices are followed. HBV infection has

been linked to multiple-use heparin vials. As with other modes of transmission, high viral titers in serum have been related to an increased risk of transmission. HBV remains infectious in the environment for 7 days or longer, so that contaminated surfaces may account for transmission in the absence of a known exposure.

SEROLOGICAL AND VIROLOGIC MARKERS

After a person is infected with HBV, the first virologic marker detectable in serum within 1-12 weeks, usually between 8-12 weeks, is HBsAg. Circulation HBsAg precedes elevation of serum aimnotransferease activity and clinical symptoms by 2-6 weeks and remains detectable during the symptomatic phase of acute hepatitis B and beyond. HBsAg becomes undectable 1-2 months. After HBsAg disappears, antibody to HBsAg becomes detectable in serum and remains detectable indefinitely thereafter. Because HBcAg in instracellular and, when in the serum, sequested within an HBcAg coat, naked core particles do not circulate in serum and therefore, HBcAg is not detectable routinely in the serum of patients with HBV infection. By contrast, anti – HBc is readily demonstrable in serum, beginning within the first 1-2 weeks after the appearance of HBsAg and preceding detectable levels of anti – HBs by weeks to months.



HBV infection can be distinguished by determination of the immunoglobulin class of anti – HBc. Anti HBc of the IgM class (IgM anti – HBc) predominates during the first 6 months after acute infection, whereas IgG anti – HBc is the predominant class of anti – HBc beyond 6 months. Therefore, patients with current or recent acute hepatitis B, including those in the anti – HBc windows, have IgM anti – HBc in their serum. In patients who have recovered from hepatitis B in the remote past as well as those with chronic HBV infection, anti – HBc is predominantly of the IgG class.

The other readily detectable serologic marker of HBV infection, HBcAg, appears concurrently with or shortly after HBsAg. Its appearing coincides temporally with high levels virus replication and reflects the presence of circulating intact virions and detectable HBV DNA.

In chronic HBV infection, HBsAg remains detectable beyond 6 months, anti- HBc is primarily of the IgG class, and anti – HBs is either undetectable or detectable at low levels. During early chronic HBV infection, HBV DNA can be detected both in serum and in hepatocyte nuclei, where it is present in free or episomal form. This replicative stage of HBV infection is the time of maxminal infectivity and liver injury; HBeAg is a qualitative marker and HBV DNA a quantitative marker of this replication phase during which all three forms of HBV circulate, including intact virions. Over time the replicative of chronic HBV infection gives way to a relatively non-replectative phase.

This occurs at a rate of ~ 10% per year and is accompanied by seroconversion from HBeAg – positive to anti – HBe positive. In most cases this seroconversion coincides with a transient acute hepatitis like elevation in aminotransferase activity, believed to reflect cell immune clearance of virus infected hepatocytes. In the non-replicative phase of chronic infection when HBV DNA is demonstrable in hepatocyte nuclear,

it tends to be integrated into the host genome. HBV replication can be detected a level of ~ less than 10^3 virions with highly sensitive amplification probes such as the polymerase chain reaction (PCR) ; below this replection threshold liver injury and infectivity of HBV are limited to negligible. Because of IgM anti-HBc, can reappear during acute exacerbations of chronic hepatitis B, relaying on IgM anti – HBc versus IgG anti – HBc to distinguish between acute and chronic hepatitis B infection, respectively, may not always be reliable, in such cases, patient history is invaluable in helping to distinguish de novo acute hepatitis B infection from acute exacerbation of chronic hepatitis B infection.

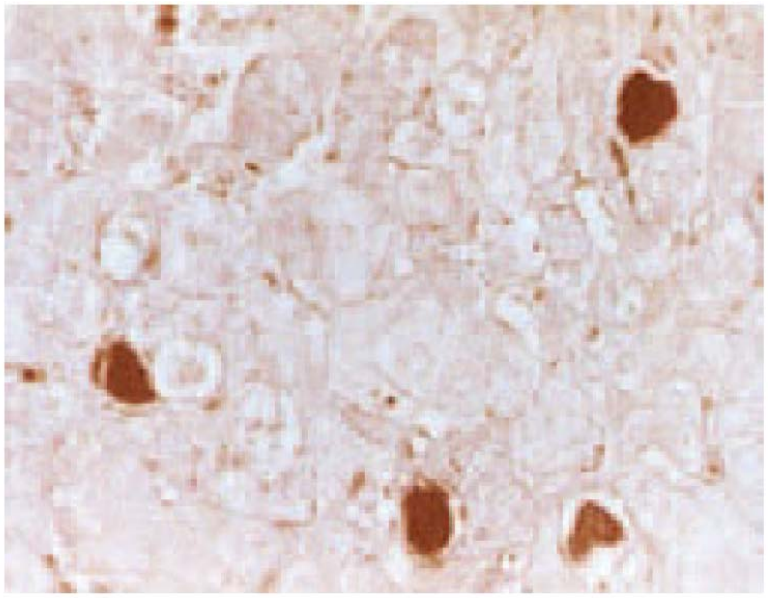


Fig. 17.10. Orcein staining shows liver cells containing HBsAg (brown).

Groups in which acute and chronic type B hepatitis should be suspected
Immigrants from Mediterranean countries, Africa or the Far East
Drug abusers
Homosexual men
Neonates of HBsAg-positive mothers
Hospital staff
Patients with
renal failure
reticuloses
cancer
organ transplants
Staff and patients of hospitals for the mentally retarded
Post-transfusion

HBV CARRIERS

Approximately 10% of patients contracting hepatitis B as adults and 98% of those infected as neonates will not clear HBsAg from the serum within 6 months. Such patients become carriers and this is likely to persist. Reversion to a negative HBsAg is rare, but may develop in old age. Males are six times more likely to become carriers than females. The dilemma of a person, such as a hospital worker, carrying the antigen and coming from an area where it is prevalent is a very difficult one. Hospital staff who develop HBsAg-positive hepatitis and clear the antigen from the blood are immune to type B hepatitis. If they become carriers, the position is difficult.'Healthy' carriers may show changes on liver biopsy ranging from non-specific minimal abnormalities through to chronic hepatitis and cirrhosis. The extent of the changes is not reflected by serum biochemical tests and may only be revealed by liver biopsy. The carrier presenting by chance is likely to have minor hepatic changes compared to the patient presenting to a gastroenterology department where more serious liver disease is probable. In a survey of patients found to be HBsAg positive at blood donation, 95% had near normal liver biopsy.

PATHOGENESIS

Immune Pathogenesis

Clinical observations suggest that the immune response of the host is more important than viral factors in the pathogenesis of liver injury caused by HBV. Chronic HBV carriers who have normal liver enzyme levels and normal or near-normal liver histologic studies, despite high levels of viral replication, have been well described; significant liver injury would be predicted if the virus were directly cytopathic. Similarly, HBV can be grown in hepatocyte culture with no adverse effect on cell viability. Other clinical observations point to the importance of an intact immune response in mediating liver injury. Infants with immature immune systems who acquire HBV infection at birth have a high rate of chronic infection and replication yet typically have only mild liver injury. Conversely, HBV-induced fulminant hepatic failure is associated with a vigorous immune response, low serum levels of virus, and massive hepatocellular necrosis.

There has been extensive investigation in both humans and experimental animals of specific immune responses associated with HBV clearance (or conversely persistence) and liver injury. In acute infection, a specific immune response to multiple viral antigens can be demonstrated

in both major histocompatibility complex (MHC) class II–restricted and MHC class I–restricted T cells ($CD4^+$ and $CD8^+$, respectively, the latter making up the majority of CTLs). T helper cell responses to core and polymerase proteins are particularly strong, with lesser responses to the envelope proteins. In patients with chronic HBV infection who fail to clear the virus, the number of both $CD4^+$ and $CD8^+$ T cells is markedly reduced. In contrast, the humoral immune response is preserved in both acute and chronic HBV infection, although anti-HBs usually cannot be detected in the latter because of antigen excess.

There are few animals models with which to investigate the pathogenesis of liver injury and the mechanisms of viral clearance in hepadnaviral infections. Transgenic mouse models have been used to examine specific mechanisms by which CTLs clear virus and cause hepatocyte necrosis. These mice are naturally tolerant to the "transgene," that is, to different components of the HBV genome, yet their immune response can be reconstituted with infusion of CTLs from non-transgenic mice immunized with different HBV proteins. Acute liver injury associated with CTL infiltration of the liver develops in a dose-dependent manner in recipients of these cells.

It has been widely accepted that CTLs are responsible for destruction of virally infected hepatocytes and for viral clearance. However, the number of CTLs involved is generally much fewer than the number (10^{11}) of virally infected hepatocytes. Thus, secondary non–antigen-specific immune responses, such as those mediated by inflammatory cytokines, may be more important for viral clearance than the CTL-mediated mechanism. Recent data point to the importance of tumor necrosis factor-α (TNF-α) and gamma-interferon as prime mediators of this non–antigen-specific clearance of HBV. These cytokines activate two independent pathways that result in elimination of HBV nucleocapsid cores and destabilization of viral RNA. These cytokines may also be important in the clearance of HBV in chronically infected patients who become superinfected with other hepatotropic viruses.

VIRAL PATHOGENESIS

Variant Viruses

Although there are a variety of serotypes and genotypes of HBV, there is remarkably little genomic variability in this virus. Nevertheless, mutant forms of HBV with mutations in the precore, surface, and X genes, as well as the core promoter region, have been implicated in a

number of clinical syndromes. Polymerase variants that are selected by exposure to nucleoside analogues have recently been described. These polymerase variants may be less replication-competent and even less pathogenic than the wild-type virus and are discussed in greater detail in the section on treatment. Several recent review articles describe the clinical significance of HBV variants and suggest changes in the nomenclature of the mutations associated with these variants.

PRECORE/CORE VARIANTS

Although the majority of Northern European and American patients with chronic HBV infection and active viral replication are HBeAg positive, many Southern European and Asian patients have severe liver disease and active viremia in the absence of HBeAg. Sequence analysis of the precore region of HBV isolated from such patients has revealed a point mutation (G to A) at nucleotide position 1896 that results in the production of a translational stop codon predicted to stop translation of HBeAg. In contrast, production of core peptide continues because translation of this protein is initiated at a start codon downstream from the site of mutation. Such variant viruses have also been described in association with fulminant liver failure and severe chronic liver disease. Whether these precore mutants are etiologically

important in these syndromes or whether they merely emerge in association with severe disease remains controversial. Moreover, if the precore mutants are etiologically associated with these syndromes, the mechanism by which the virus causes these syndromes is unknown. Several hypotheses have been put forward, including 1) a heightened immune response against core peptide expressed on hepatocytes in the absence of "immune deflection" by HBeAg in serum (see previous section); 2) direct cytopathicity of the truncated HBeAg fragment that is synthesized as a result of early termination of translation of precore/core mRNA; 3) enhanced replication of mutant virus over wild-type virus because of increased stability of the hairpin stem-loop of the HBV genome, which encodes the encapsidation signal for viral packaging. In the United States, only a small proportion of all cases of HBsAg-positive fulminant liver failure is associated with variant viruses.

Mutations in the core promoter region have also been proposed to result in failure of HBeAg production. These mutant viruses, which involve nucleotide substitutions at positions 1762 and 1764, may decrease transcription of mRNA that encodes HBeAg. Core promoter variants have been described in association with 10% of cases of fulminant HBV

infection, 10% of cases of acute self-limited hepatitis, and 27% of cases of progressive chronic hepatitis.

Although there has been a great deal of interest generated by the discovery of HBV variants, the clinical significance of these mutant viruses, particularly those described in association with severe liver disease, remains controversial. The presence of precore variants is also associated with the distribution of HBV genotypes. In patients with genotype A HBV infection (the predominant genotype in North America and Europe), nucleotide (nt) 1858 is a C. In this genotype, both a mutation at nt 1896 (G to A) and nt 1858 (C to T) would be required to stabilize the stem-loop structure of the HBV genome. Without a compensatory mutation at nt 1858 in genotype A, impaired base pairing results when C-1858 tries to pair with A-1896, thereby destabilizing the stem-loop structure of the packaging signal and leading to a decrease in encapsidation and in viral replication. Thus, precore stop codon mutations may be less frequent in genotype A because of the need for two mutational events. By contrast, in countries where precore mutants are common, genotypes B, C, D, and E are predominant. In these patients only one mutation at nt 1896 is required to yield a precore mutant with stable stem-loop pairing of the genome.

SURFACE GENE MUTANTS

Immune escape from neutralizing antibodies occurs in a region of HBV known as the "a" determinant. This hydrophilic region from amino acids 124 to 147 is highly conserved between subtypes of HBV and is believed to be important in eliciting protection against infection. The glycine at amino acid 145 in particular is highly conserved among HBV subtypes, and a G-to-A nucleotide substitution produces amino acid changes at this position that result in major antigenic changes in the virus. Escape mutants have been described in babies who have received immunoprophylaxis with polyclonal hepatitis B immune globulin (HBIG). Despite protective levels of antibody, these babies became HBsAg positive and developed chronic liver disease. Virus from such a baby demonstrated a G-to-A substitution at amino acid 145 compared with the viral isolate from the mother, and the mutant virus bound HBIG with lower affinity than did the "wild-type" virus. Emergence of surface gene mutants in liver transplant recipients receiving monoclonal HBIG therapy has also been described. As with the "vaccine-escape" mutants, these viruses fail to bind anti-HBs with high affinity. Because of the very compact nature of the genomic organization of HBV, changes in the "a" determinant have the potential to alter the function of the HBV

polymerase, an enzyme essential for viral replication. The G-to-A mutation in the "a" determinant may give rise to a stop codon (TAG) (if the nucleotide before this mutation is a T), which would have profound effects on viral replication and the pathogenesis of disease.

LOW-LEVEL HBV INFECTION

The existence of HBsAg-negative HBV infection has long been debated. Low-level HBV infection is implicated or has been documented in the following clinical situations: a) HBV infection has been transmitted by transfusion of a unit of blood from an HBsAg-negative, anti-HBc-positive blood donor; b) HBV DNA has been detected in serum and liver from persons lacking all serologic markers of HBV infection. Serum from serologically negative, HBV DNA–positive persons can transmit HBV infection to chimpanzees; c) occult HBV infection has been reported in association with apparent fulminant hepatic failure in patients who lack all serologic markers of HBV or HAV infection (so-called non-A, non-B fulminant hepatic failure), although these results are controversial ; d) low-level HBV infection has been detected in patients with a variety of causes of chronic liver disease and in association with primary hepatocellular carcinoma. Even in regions of low endemism for HBV, such as France, 11 of 22 HBsAg-negative patients with hepatocellular

carcinoma had detectable HBV DNA in serum. Molecular studies have demonstrated selective accumulation of RNA encoded by the X gene in association with HBsAg-negative hepatocellular carcinoma) HBsAg-negative patients may become HBsAg positive and develop overt hepatitis with cancer chemotherapy or immunosuppression following kidney transplantation. Presumably low-level HBV infection is the source of viral reactivation that is unmasked by immunosuppression.

Whether viral or host immune factors enable persistence of HBV infection in the absence of HBsAg is unclear. Two hypotheses have been proposed to explain the atypical serologic profiles in these patients. The first hypothesis suggests that mutations in the viral genome result in impaired antigen production by the virus. Numerous mutations in HBV, particularly in the region of the gene for HBsAg, have been reported and may facilitate viral persistence. The second hypothesis suggests that the host immune system keeps the virus in a quiescent or latent state until immunosuppressive therapy results in viral reactivation.

CLINICAL MANIFESTATIONS

Acute Infection

The incubation period from acute exposure to clinical symptoms ranges from 60 to 180 days . Clinical presentation varies from asymptomatic infection to cholestatic hepatitis with jaundice, and rarely liver failure. In acute infection, HBsAg and markers of active viral replication (HBeAg and HBV DNA by hybridization assays) become detectable approximately 6 weeks after inoculation, before the onset of clinical symptoms or biochemical abnormalities. These tests remain positive throughout the prodromal phase and during the early clinical phase of the illness. Biochemical abnormalities usually coincide with the prodromal phase of the acute illness and may persist for several months. With the onset of symptoms, IgM anti-HBc becomes detectable. IgM anti-HBc may persist for many months, and IgG anti-HBc may persist for many years, if not a lifetime. Anti-HBs is the last serologic test to become positive and is a marker of resolving infection (as HBsAg titers fall). Much has been made of the serologic window period when neither HBsAg nor anti-HBs is detectable and IgM anti-HBc is the only marker of acute infection. However, with currently available serologic assays, this window period is rare.

The biochemical diagnosis of acute hepatitis depends largely on measurements of serum bilirubin and aminotransferase levels. The serum alanine aminotransferase (ALT) level is typically higher than the serum aspartate aminotransferase (AST) level, and levels of both aminotransferases are usually 500 U/L or greater. Bilirubin elevations are usually modest (5–10 mg/dL), although they may be higher in the setting of hemolysis or renal failure.

The most profound complication of acute HBV infection is fulminant hepatic failure, defined as the onset of hepatic encephalopathy within 8 weeks of the onset of symptoms. Although this complication is infrequent (occurring in less than 1% of cases), the prognosis is poor once encephalopathy has developed. When patients present with acute hepatitis, it is essential to obtain tests of hepatic synthetic function (prothrombin time, serum albumin). Evidence of a prolonged prothrombin time (International Normalized Ratio [INR] of 1.5 or greater or prothrombin time of 17 seconds or longer) should raise concern regarding the potential development of fulminant hepatic failure. If clinical symptoms of hepatic failure develop, patients should be referred for consideration of liver transplantation.

Current serologic assays for the diagnosis of acute and chronic HBV infection (HBsAg and HBeAg) are both sensitive and specific. HBsAg, HBeAg, anti-HBc, and anti-HBe are detected by standardized enzyme-linked immunoassays (EIAs). The detection of HBsAg indicates active HBV infection, and the detection of HBeAg indicates active viral replication and increased infectivity. Characteristic serologic changes develop in relation to clinical symptoms and biochemical abnormalities in acute resolving infection and in acute followed by chronic infection.

CHRONIC INFECTION

In acute HBV infection that progresses to chronicity, early events are similar to those in acute HBV infection that resolves. However, with chronic infection, HBsAg, HBeAg, and HBV DNA remain positive for 6 months or longer. After the acute phase of infection, serum ALT levels fall but often remain persistently abnormal (from 50 to 200 U/L). IgM anti-HBc titers typically fall to undetectable levels after 6 months but may become detectable again during reactivation of infection. IgG anti-HBc persists indefinitely. HBV DNA is detectable by hybridization assays during the acute and chronic phases of disease. With time, there may be a spontaneous loss of HBV DNA and HBeAg, frequently in association with a flare of serum ALT levels and seroconversion to anti-

HBe positivity. Spontaneous loss of HBsAg is rare. Anti-HBs may be detected simultaneously with HBsAg in serum in fewer than 10% of cases. In some cases of chronic infection, active viral replication (HBV DNA positivity) occurs in the absence of HBeAg (see previous section).

The presence of anti-HBs is associated with immunity to HBV infection. Isolated anti-HBs is more likely to be acquired by vaccination than by natural infection, in which both anti-HBs and IgG anti-HBc are typically present. Much confusion surrounds the interpretation of isolated anti-HBc positivity. The significance of this finding depends on the patient population in which it is observed. Fifty percent of patients with chronic HCV infection are anti-HBc positive, and in these patients, who have had frequent parenteral exposure, the anti-HBc positivity likely represents resolved HBV infection or low-level HBV infection of minor clinical significance. In Alaskan natives, a group with a high prevalence of HBV infection, vaccination against HBV has been used to determine whether isolated anti-HBc signifies prior exposure; those with a primary immune anti-HBs response were deemed to have a false-positive anti-HBc, whereas those with an anamnestic response were deemed to have a true-positive anti-HBc, indicative of prior or ongoing low-level HBV infection. In blood donors who have been pre-screened for parenteral risk

factors, an isolated anti-HBc, particularly if it is of low optical density on EIA, likely represents a false-positive result. Because there is no readily available "gold standard" for the diagnosis of resolved or ongoing low-level HBV infection, the interpretation of an isolated positive anti-HBc remains problematic

Extrahepatic Manifestations

Extrahepatic findings are common in patients with acute HBV infection. Arthralgias and rashes occur in 25% of cases. A severe serum sickness–like syndrome with immune complex deposition occurs more rarely and can result in angioneurotic edema. Polyarteritis nodosa with a systemic vasculitis can occur with either acute or chronic HBV infection. This syndrome typically presents with abdominal pain resulting from arteritis of the medium-sized arteries with ischemia to the intestine or gallbladder. Other manifestations of HBV-associated vasculitis include neuropathy (mononeuritis), renal disease, cutaneous vasculitis, arthritis, and Raynaud's phenomenon. Chronic, and to a lesser extent acute, HBV infection has also been associated with membranoproliferative glomerulonephritis resulting from deposition of immune complexes in the basement membrane of the glomerulus. The syndrome of type II mixed essential cryoglobulinemia was previously believed to be caused largely

by HBV infection but has been shown to be associated more frequently with chronic HCV infection (see later section). Neurologic manifestations of HBV infection include Guillain-Barré syndrome and a polyneuropathy (usually related to polyarteritis). HBV infection is rarely associated with pericarditis and pancreatitis.

COMPLICATIONS

Patients with chronic HBV infection are at risk of developing long-term complications of portal hypertension and hepatic decompensation, such as variceal bleeding, ascites, and hepatorenal syndrome, as well as HCC, which may ultimately result in death. When complications occur, referral for liver transplantation should be considered. Evolving antiviral therapies, discussed later, have greatly improved the outcome of patients with HBV infection after liver transplantation.

Patients with chronic liver disease, particularly those with established cirrhosis, are at increased risk of developing HCC. The risk of developing HCC is increased 10- to 390-fold in patients with chronic HBV infection compared with those who are HBsAg negative and is greater in those who acquired HBV infection perinatally than in those who acquired the infection as adults. In regions where HBV is endemic, HCC is the leading cause of cancer-related deaths.

Despite this strong epidemiologic link between HBV infection and HCC, and despite active research in this area for more than a decade, the mechanism by which HBV causes malignant transformation has not been elucidated. Comparison of HBV DNA sequences with those of known oncogenes has failed to identify a specific viral oncogene. Activation of an adjacent host cellular oncogene may occur during the process of HBV genome integration, but the viral genome does not integrate into the host genome in any consistent pattern. Cirrhosis of the liver is present in more than 90% of patients with HCC related to HBV, suggesting that the presence of cirrhosis is a risk factor for HCC development. Chronic inflammation associated with active viral replication, together with ongoing cellular proliferation and regeneration associated with cirrhosis, is likely a predisposing factor that leads to cellular transformation and frank malignancy.

LABORTORY FEATURES

The serum aminotransferases aspartate aminotransferase (AST) and ALT show a variable increase during the prodromal phase of acute viral hepatitis precedes the rise in bilirubin level.

The acute level of these enzymes, however, does not correlate well with the degree of liver cell damage. Peak levels vary from 400 – 4000 IU or more, these levels are usually reached at the time the patient is clinically icteric and diminish progressively during the recovery phase of acute hepatitis. The diagnosis of anicteric hepatitis is based on clinical features and on aminotransferase elevations.

The serum bilirubin may contribute to the rise despite falling serum aminotranferase levels. In most instances, the total bilirubin is equally divided between the conjugated and unconjugated fractions. Bilirubin level > 340 umol/L (20mg/dL) extending and persisting late into the course of viral hepatitis are more likely to be associated with severe disease.

Neutropenia and lymphopenia are transient and are followed by relative lymphocytosis. Atypical lymphocytes are common during the acute phase. Measurement of prothrombin time is important with acute viral hepatitis for a prolonged value may reflect a severe hepatic synthetic defect, signify extensive hepatocellular necrosis, and indicate a worse prognosis.

Serum IgG and IgM levels are elevated in about one – third of patients during the acute phase of viral hepatitis but the serum IgM level is elevated more characteristically during acute hepatitis.

Diagnosis of HBV infection can usually be made by detection of HBsAg in the serum. Infrequently levels of HBsAg are too low to be detected during acute HBV infection even with contemporary, high sensitive immunoassays. In such cases the diagnosis can be established by the present of IgM anti- HBc.

The titer of HBsAg bears little relation to the severity of clinical disease. Indeed, an inverse correlation exists between serum concentration of HBsAg and the degree of liver cell damage. For example titers are highest in immunosupperssed patients, lower in patient with chronic liver disease and very low in patients with acute fulminant hepatitis. These observation suggest that, in hepatitis B, the degree of liver damage and the clinical course are related to variations in the patients immune response to HBV rather than to the amount of circulating HBsAg. Immunocompetent persons, however, there is a correlation between markers of HBV replication and liver injury.

Another serologic marker that may be value in patients with hepatitis B is HBeAg. Its principal clinical usefulness is as an indicator of relative infectivity. Because HBeAg is invariably present during early acute hepatitis B, HBeAg testing is indicated primarily during follow up to chronic infection.

Anti – HBs is rarely detectable in the presence of HBsAg in patients with acute hepatitis B, but 10-20% of person with chronic HBV virus infection may harbor low – level anti – HBs. This antibody is directed not against the common group determinant, but against the heterotypic subtype determinant, in most cases this serological pattern cannot be attributed to infection with two different HBV subtypes, and the presence of this antibody is not harbinger of imminent HBsAg clearance.

After immunization with hepatitis B vaccine, which consists of HBsAg alone, anti – HBs is the only serologic marker to appear.

Like HBeAg, serum HBV DNA is an indicator of HBV replication, but tests for HBV DNA are more sensitive and quantitative.

Currently, testing of HBV DNA has shifted from insensitive hybridization assays to amplification assays, example PCR based assay, which can detect as few as 10-100 virions/ml ; among the commercially available PCR assays, the most useful are those with high sensitive (5-10 IU/mL) the largest dynamic range (10^{o}-10^{9} IU/ml).

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

This study was undertaken to establish the relationship between biochemical, serological, histological and viral replication indices in an individual with incidentally detected asymptomatic HBsAg positive.

MATERIALS AND METHODS

MATERIAL AND METHODS

This study was undertaken in 112 asymptomatic subjects incidentally detected to be HBsAg positive with ALT levels less than five the upper limit of normal, attending the Department of Medical Gastroenterology, COIMBATORE MEDICAL COLLEGE HOSPITAL, COIMBATORE between September 2007 to September 2009. Their

clinical, biochemical, virological and histological assessment was done. 39 patients were lost on follow up.

INCLUSION CRITERIA :

1. HBsAg positivity on 2 occasions.
2. IgM Anti-HBc negative.
3. ALT (<5 times normal).

EXCLUSION CRITERIA:

1. History of jaundice <1 year.
2. Presence of Ascitis.
3. GI Bleeding .
4. Hepatic Encephalopathy.
5. Pregnant women.
6. Those with immunodeficiency (renal/bone marrow transplant recipients or those on cancer chemotherapy / Hemodialysis.
7. Co infection with HIV or Hepatitis C.
8. Alcohol consumption ≥20 gm/day for >5 year.

9. The study was approved by the ethics committee of Coimbatore Medical College Hospital, Coimbatore.

METHODOLOGY

METHODOLOGY

All the subjects were interviewed on the basis of a pre designed questionnaire regarding occupation, exposure to unsterilised needle and syringes. Blood transfusion, post history of Jaundice (etiology and outcome) any family member suffering from Hepatitis B/C/E, history of ingestion of any hepatotoxic drugs, alcohol injection (amount, since how many year), any surgical procedure, high risk behaviors as intravenous drug abuse, sexual promiscuity, family history of chronic liver disease, tattooing shaving razor, blade etc. none of the patients had a history of Hemodialysis or organ transplantation. Detailed general examination was carried out to look signs of chronic liver disease.

All the subjects serum were tested for serum Bilirubin, ALT,AST, HBeAg, IgM anti-HBc, anti HCV, HIV, and HBV DNA (quantitative by PCR)

The subjects were divided into following groups :Group A(6):HBeAg positive,HBV DNA $>10^5$, ALT $\geq$40 (ie 40IU/ml).Group B(4):HBeAg positive, HBV DNA $>10^5$, ALT < 40.Group C(12):HBeAg negative, HBV DNA $\geq$ 10000 copies/ml, ALT $\geq$ 40.Group D(16):HBeAg

negative, HBV DNA ≥ 10000 copies/ml, ALT<40.Group E(35):HBeAg negative, HBV DNA <10000 copies/m, ALT <40

Liver biopsy for HAI scoring was done in group A, B, C and D individuals, after taking written informed consent.

Biochemical parameter (ALT, AST, Serum Bilirubin)were measured using an auto analyser. Prothrombin time was measured using Quick method at the time of Liver biopsy.

The sera of the subjects were tested for HBsAg, IgM HBcAg, HBeAg and Anti-HBe (VIDAS automated module using enzyme linked Fluorescent immunoassay [ELFA], biomerieux Sa, France) anti HCV (3rd generation ELISA Kit, ELISCAN™ HCV), HIV I and II and p24 [MINI VIDAS (ELFA)] and HIV DUO ULTRA (HIV5).

Test for HBV DNA was done in sera of all subjects from using "Real Time Polymerase Chain Reaction". It involves the specific amplification of a 134bp region of the HBV genome. This analysis is done on Rotor Gene 3000 by using the highly sensitive & specific TAQMAN assay method. The TAQMAN probes are used for fluorescent detection of only target sequence specific amplification generated during PCR. Amplified products are indicated by Threshold Cycle (Ct) in

amplification curve. The analytical detection limit of the test is 3.8 IU/ml (26.6 copies/ml).

Liver biopsy was performed percutaneously using a 16 G Tru Cut needle after attaining an informed consent from patients in group A, B, C and D whenever coagulation profile permits. An adequate liver biopsy was defined as one in which three or more portal tracts were present. HAI Scoring using Modified Ishak's Scoring for grading and staging of liver disease. Two individuals refused to give consent for liver biopsy.

TABLES

TABLE 1 : COMPARISON OF HBV DNA

LEVEL,ALT,AST,GRADE AND STAGE IN CASES WITH

HBeAg POSITIVE & NEGATIVE:

Factor	HBeAg	N	Mean	Std. Deviation	Std. Error Mean	P
HBV DNA (copies/ml)	Positive	10	14910410.00	7102572.426	2246030.611	<0.05
	Negative	44*	244983.68	587039.604	88499.550	
AGE (in years)	Positive	10	27.5000	8.61846	2.72539	>0.05
	Negative	63	33.6032	11.79330	1.48582	
ALT (IU/ml)	Positive	10	59.20	27.259	8.620	<0.05
	Negative	63	32.77	13.959	1.759	
AST(IU/ml)	Positive	10	50.9000	24.76310	7.83078	<0.05
	Negative	63	26.4730	10.06055	1.26751	
GRADE**	Positive	10	5.70	1.494	0.473	<0.05
	Negative	29	3.34	0.936	0.174	
STAGE**	Positive	10	2.10	1.101	0.348	>0.05
	Negative	29	0.69	0.761	0.141	

*HBV DNA was Negative i.e. (<26.6 copies/ml) in 19 cases.

**Liver biopsy was done in 39 cases.

TABLE 2: SHOWING NUMBER OF CASES, MEAN AGE, ALT, AST, HBV DNA GRADE AND STAGE IN EACH GROUP:

Group	Frequency	Mean ALT (IU/ml)	Mean AGE	HBV DNA Mean (Copies / ml)	Mean AST (IU/ml)	Grade	Stage
A	6	80.00	25.6667	16741900.00	68.0000	5.83	2.50
B	4	28.00	30.2500	12163175.00	25.2500	5.50	1.50
C	14	54.54	32.7857	184937.36	41.3714	3.36	0.86
D	15	26.73	38.667	543018.27	23.0000	3.69*	0.62
E	34	26.47	31.7059	2992.33**	21.8706	1.00***	0***
Total	73	36.39	32.7671	2960803.37	29.8192	3.95	1.05

*Liver Biopsy was done in 13 cases of Group D.

**HBV DNA was Negative i.e. (<26.6 copies/ml) in 19 cases of Group E.

***Liver biopsy was done in 2 cases of Group E.

TABLE 3 : COMPARISON OF ALT, AST, HBV DNA Levels, GRADE AND STAGE

Group	ALT(P value)	HBV DNA [P value]	AST [P value]	Grade P value	Stage P value
C&D	P < 0.05	P >0.05	P >0.05	P >0.05	P > 0.05
C&E	P <0.05	P<0.05	P >0.05	N/A	N/A
D&E	P > 0.05	P < 0.05	P >0.05	N/A	N/A

TABLE 4: COMPARISON OF CASES WITH ELEVATED AND NORMAL ALT WITH HBV DNA LEVELS, GRADE, STAGE AND AST:

	ALT	N	Mean (copies/ml)	Std. Deviation (copies/ml)	Std. Error Mean (copies/ml)	P
DNA	≥ 40	20	5152026.15	8188966.486	1831108.573	>0.05
	< 40	34	1671848.79	4907189.807	841576.109	
GRADE	≥ 40	20	4.10	1.518	0.340	>0.05
	< 40	19	3.79	1.512	0.347	
STAGE	≥ 40	20	1.35	1.182	0.264	>0.05
	< 40	19	0.74	0.806	0.185	
AST (IU/ml)	≥ 40	20	49.3600	15.67104	3.50415	>0.05
	< 40	53	22.4453	5.81585	0.79887	

TABLE 5: CORELATION OF HBV DNA LEVEL, ALT AND AST WITH EACH OTHER AND WITH LIVER HISTOLOGY (HAI SCORE):

FACTOR	HBV DNA (P value)	ALT (P value)	AST (P value)
GRADE	<0.001	>0.05	<0.05
STAGE	<0.001	>0.05	<0.05
ALT	<0.001	-	-
AST	<0.001	-	-
DNA	-	>0.05	<0.05

RESULTS

RESULTS

Statistical analyses were carried out using the statistical program for social sciences (SPSS). Pearson correlation was carried out. Two tailed value of less than 0.05 was considered statistically significant. Independent t-test was carried for testing the significance between two variables. Results are presented as mean $\pm$ SD.

A total of seventy three cases(57 males and 16 females with mean age of 32.84$\pm$10.23 years and 32$\pm$15.81 years respectively) cases were included in the study, as they could complete the desired protocol .

In our study Ten cases were HBeAg positive (13.7%)and HBV DNA positive and sixty-three were HBeAg negative (86.3%) individuals of whom nineteen (26 %)cases were HBV DNA negative (<26.6 copies/ml). There was statistically significant difference in grade, HBV DNA levels and ALT but, there was no significant difference in the stage and age between the two groups.

In our study there were 6 (8.2%) individuals in group A, 4 (5.5%) in group B, 14 (19.2%)in group C, 15 (20.5%) in group D and 34(46.6%) individuals in group E. There was no statistically significant difference between the Groups C and D in HBV DNA levels, AST, Stage and Grade

(in Liver biopsy), though there was statistically significant difference in ALT levels between the 2 groups. Also there was statistically significant difference in ALT levels between cases in Group C and E. There were significant difference in DNA levels between group D & group E and between group C & group E. Other groups were not tested for their statistical significance as there were too few in number.

HBV DNA levels ranged from <26.6 to 2.5 x 10^7 copies/ml.

All the individuals were negative for IgM HBcAg, HIV and anti-HCV.

The ALT levels ranged from 14to 85 IU/ml. There were twenty cases with elevated ALT i.e. $\geq$ 40 IU/ml and thirty-four cases with normal ALT i.e. <40 IU/ml. There was NO statistically significant difference in HBV DNA levels, grade and stage in cases with elevated and normal ALT. There was no correlation of ALT with HBV DNA, Grade and Stage.

The AST level ranged from 13 to 92 IU/ml. There was significant co-relation of AST with HBV DNA levels, grade and stage of liver disease

HBV DNA levels Co-related significantly with grade i.e. level of inflammation in Liver biopsy ($r = 0.48$, $p < 0.001$) [TABLE 10]. HBV DNA levels also co-related statistically with Stage i.e. fibrosis score($r = 0.47$, $p < 0.001$).There was also statistically significant co-relation of ALT ($r = 0.37$, $p < 0.001$) and AST ($r = 0.42$, $p < 0.001$) with HBV DNA levels. There was no correlation between HBV DNA levels with Age of the patient.

DISCUSSION

DISCUSSION

Hepatitis B is an important cause of Acute and chronic liver disease in the world. The aim of this study was to determine the relationship between biochemical, serological, histological and viral replicative indices in an incidentally detected asymptomatic HBsAg positive.

The Natural history of HBsAg carrier is in Asian Countries is different from that of the Western Countries. In the west a number of studies have demonstrated the benign outcome of HBsAg carries Manno et al [4], Reinicke et al [5], Koretz et al [6], Villeneuve et al[7], Dragosiscs et al [8], de Francis et al [9]. This favorable outcome hasn't been shared by similar studies conducted in the Asian region Will et al [10], Hsu et al [11], Beasly et al [12], Obata et al [13].

In the present study there were 10 HBeAg positive constituting 13.7% of the total study group with a mean age of 27.5$\pm$8.62 years and 63 HBeAg negative individuals constituting 86.3% of the total with a mean age of 33.6$\pm$11.79 years. Though difference in age was present, similar to other studies in which HBeAg negative individuals were older than HBeAg positive, the difference was not statistically significant in the present study group. This is in discordance with earlier studies by Jie et al [14] Manesis et al [15], Yuen et al [16], Chan et al [17], who all had demonstrated

that HBeAg negative cases are older than HBeAg negative. This may be due to the small number of cases in the present study.

In the present study HBV DNA was negative in 19 patients while in the remaining fifty-four cases had HBV DNA ranging from 119 to 2.5 x 10^7 copies/ml. The wide variation in HBV DNA levels could be explained by that HBV isolates have different genetic subtypes, quesispecies, viral mutation and different immune ability to HBV Yao et al [18]

In the present study HBV DNA levels were strongly correlated with liver histology in both HBeAg positive and HBeAg negative cases.[With grade r = 0.48, p <0.001 (Significant); stage, r = 0.48, p <0.001 (significant)].Along with this there was significant correlation with ALT (r=0.37, p <0.001), and AST (r = 0.42, p <0.001) Mommeja et al [19], Yuen et al [20] Iloeje et al.[21] But this is in discordance with Huo et al [22], Chandra et al [23], Lok et al [24] and Jie et al [14], Kendall et al [25] who all demonstrated that, Liver histology was correlated with ALT level and not with HBV DNA levels.

In the present study a correlation was found between HBV DNA levels and ALT, AST in both HBeAg positive and HBeAg negative individuals. The correlation co-efficient was high. Habersetzer et al [22.]

There was no statistically significant co-relation between Alt levels with grade of liver disease.This is in discordance with earlier studies which had shown a co-relation between ALT level and grade of liver disease, Huo et al [23], Chandra et al [24], Lok et al [25] Kendall et al [26] though it is concordance with the newer studies Mommeja-Marin et al [19], Yuen et al [20], Iloeje et al.[21] .In the present AST levels strongly co-related with stage and grade which in concordance with other similar studies. Heo et al [27], Borg et al [28].

Iloeje et al [21] had shown that patients with e-antigen negative chronic HBV infection have fluctuating ALT levels and one point estimation of ALT may not be reliable. Keeffe et al [29] showed that patients with normal ALT may be associated with significant fibrosis especially in patients with a negative chronic HBV infection. ALT elevation in chronic hepatitis B patients may occur spontaneous or drug induced seroconversions or due to superadded insult with other viruses or drug. Perrilo et al [30]. With the help of the above mentioned information, it is reasonable to conclude that the ALT levels have poor predictability for progression of liver disease and planning treatment in patients with CHB.

In conclusion we can say that HBV DNA levels (PCR) is better in determining histological activity and in guiding treatment of asymptomatic HBsAg positive individuals than ALT levels.

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